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POTENTIAL OF TURMERIC EXTRACT AND ITS FRACTIONS TO CONTROL PEACH FRUIT FLY (DIPTERA: TEPHRITIDAE)

Potencial do extrato de cúrcuma e suas frações para controlar mosca do pêssego (Diptera: Tephritidae)

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ABSTRACT

Potential of turmeric extract and its chemical fractions were evaluated to control the infestation of *Bactrocera zonata* peach fruit fly in a mortality-based bioassay. The turmeric extract (TE) was taken on Soxhelt's extraction apparatus and chemically fractioned by thin layer followed by column chromatography into 6 fractions (F1 …F6). Fifty pairs of the flies were fed in cages with 250 and 500 ppm TE and its fractions separately for 20 days along with flies fed on untreated diet to serve as control. The toxicity of TE and each of its fractions was evaluated by calculating percent mortality of fly population after every 5th day in 4 consecutive intervals. Mortality of fly population was observed to be positively correlated with increasing concentrations of TE and its fractions in diet. The mortality of flies fed at 250 and 500 ppm TE was significantly higher at 44.17 and 66.33% compared to 28.88% in control. Percent mortality was much higher in case of flies fed with fractions F1, F3 and F6 i.e. 72.22, 50.00 and 48.76 respectively. Maximum rise of mortality was observed at the end of 3rd interval; in case of flies fed at 500 ppm TE, 52.45 percent mortality was observed at the end of 3rd interval; highest mortality was caused by fraction F1, 51.39% in case of flies fed at 250 ppm and 70.37% in case of those fed at 500 ppm.

Index terms: Pest control; natality; thin layer chromatography.

RESUMO

O potencial do extrato de curcuma e suas frações químicas foram avaliados para controlar a infestação da mosca do pêssego, *Bactrocera zonata* em um ensaio biológico à base de mortalidade. O extrato de cúrcuma (EC) foi obtido por aparelho de extração de Soxhelt e quimicamente fracionado em seis frações (F1 ... F6) por cromatografia em camada fina e cromatografia em coluna. Cinquenta pares de moscas acondicionadas em gaiolas foram alimentadas com 250 e 500 ppm de EC e suas frações, em separado, durante 20 dias, juntamente com moscas não alimentadas com a dieta para servir de controle. A toxicidade de EC e cada uma das suas fracções foi avaliada calculando a porcentagem de mortalidade de cada população de moscas, a cada 5 dias, em 4 intervalos consecutivos. A mortalidade de população de moscas foi, positivamente, correlacionada com as concentrações de EC e suas frações na dieta. A mortalidade das moscas alimentados em 250 e 500 ppm de EC foi, significativamente, maior em 44,17 e 66,33% em comparação com 28,88% no controle. A mortalidade foi maior no caso de moscas alimentadas com as frações F1, F3 e F6, ou seja, 72,22%, 50,00% e 48,76% respectivamente. O aumento máximo da mortalidade foi observado no final do terceiro intervalo; no caso de moscas alimentadas a 500 ppm de EC, 52,45% de mortalidade foi observada no final do terceiro intervalo. A maior mortalidade foi causada pelas frações F1, 51,39% no caso das moscas alimentadas com 250 ppm e 70,37%, no caso daqueles alimentadas com 500 ppm.

Termos para indexação: Controle de pragas; natalidade; cromatologia em camada fina.

INTRODUCTION

Peach fruit fly (*Bactrocera zonata*) is a common and widely spread specie in nearly all the fruit growing areas of the world (Dhillon et al., 2005; Grewal, 1981). Some of its major host plants include *Psidium guajava* (guava), *Mangifera indica* (mango), *Eriobotrya japonica* (loquat), *Prunus persica* (peach), *Citrus spp*. (orange), and *Diospyros lotus* (persimmon) (Goergen et al., 2011, Mwatawala, et al., 2009). The fruit flies have gained more importance with respect to WTO's quality regulations regarding sanitary and phyto-sanitary (SPS) standards, hazardous chemicals, post and pre harvest practices, export and quarantine laws (Datta et al., 2001; Enkerlin, 2007). Fruit fly infestation and its resultant consequences in the shape of pesticide residue and quality deterioration of fruits are putting adverse effects

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on the economy of farmers and traders and environment (Drew; Hancock, 1994). The global loss accrued by fruit flies infestation ranges in billions of dollars (Stonnehouse; Mumford; Mustafa, 1998).

Use of insecticides as cover spray to affected crops to prevent fruit fly damage is a common practice all over the world. Insecticides like Dipterex, Nogos, Folidol, Dimecron and Metasystox have been employed by farmers to control fruit fly infestation (Roush et al., 1986; Zhang et al., 2014), however, the insecticidal control has resulted in many environmental and collateral pest problems in agro ecosystem (Sultan et al., 2000). The potential agro-ecological and human health consequences of insecticides have led to a search for alternative pesticides that are more environment friendly (Stark; Vargas; Miller, 2004).

Turmeric, (*Curcuma longa*) a subterranean perennial and tuberous herb widely cultivated in Pakistan and India, has been reported to contain potentially toxic components for different insect pests of stored grains (Siddiqi et al., 2011). Different extracts and their chemical fractions of turmeric have been found to contain strong repellent and toxic effects against different pests of stored rice and wheat (Siddiqi et al., 2011). Acetone and petroleum ether extract of turmeric have been found to contain turmerones, which comprise of three important compounds, α-turmerone, β-turmerone and ar-turmerone (Figure 1) (He et al., 1998, Singh et al., 2010).

Turmerones have demonstrated insecticidal and insect repellent properties against *Plutella xylostella, Plodia interpunctella* and *Spodoptera litura* larvae and *Sitophilus oryzae*, *Callosobruchus chinensis* and *Lasioderma serricorne* adults; ar-turmerone has been found to exhibit repellence against *Tribolium castaneum* (Mostafa, 1993). The petroleum ether, ethanol and acetone extracts of turmeric has also been tested in mortality and growth inhibition based bioassays against peach fruit fly with acetone extract showing highly significant growth inhibition and mortality effects (Siddiqi et al., 2006). Besides turmerone and its derivatives, curcuminoids are another group of compounds extracted from turmeric which have been observed to be isolated through acetone of turmeric (Su; Robert; Jilani, 1982; Priyadarsini, 2009). Curcuminoids comprise of three closely related compounds, curcumin (also known as Diferuloylmethane, $C_{21}H_{20}O_6$), demethoxy-curcumin and bisdemethoxy-curcumin (Figure 2) (Lee et al., 2013). Curcuminoids have shown strong insecticidal activity against *Tribolium castaneum* adults, *Aedes aegyptii* larvae and *Schistocerca gregaria* and *Dysdercus koenigii* nymphs (Chowdhury; Walia; Saxena, 2000; Roth; Chandra; Nair, 1998; Su et al., 1982). The objective of this study was to evaluate the toxicity of different chemical fractions of turmeric to *Bactrocera zonata* peach fruit fly.

MATERIAL AND METHODS

Rearing Fruit Flies

Bactrocera zonata flies were reared in laboratory under controlled conditions in glass cages measuring 45 X 40 X 40 cm at room temperature 28 ± 1 ℃ and relative humidity 55 ± 5% R.H fed with artificial diet consisting of the following ingredients: two bananas, six eggs yolks, four table spoon of honey, two table spoon of Vit B.complex syrup, one table spoon of yeast and eight table spoon of sugar.

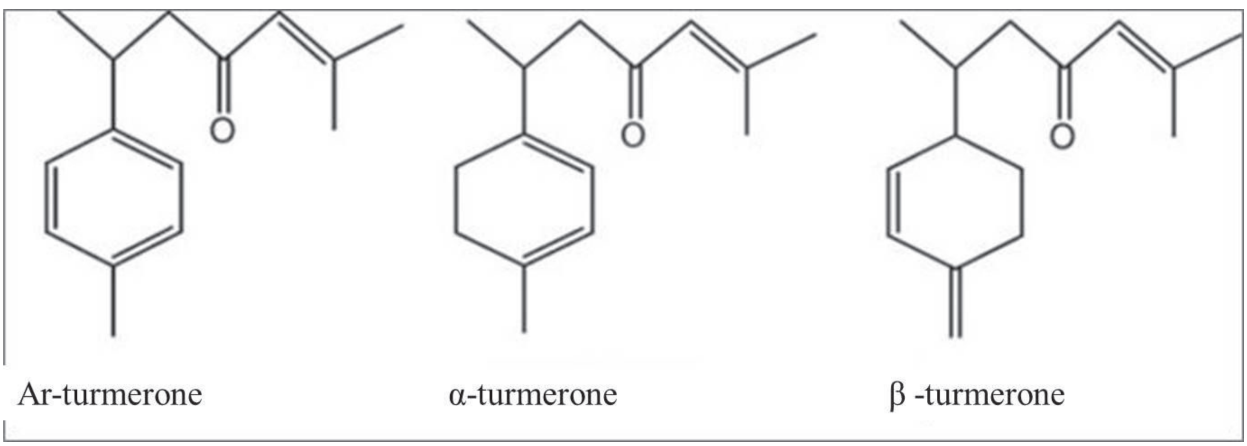

Figure 1: Structural formulae of the three major turmerone types: ar-turmerone, α-turmerone and β-turmerone.

Preparing chemical extracts and fractions

The acetone extract of turmeric was prepared from finely powdered rhizomes of turmeric in an electric grinder. A weighed amount of turmeric powder was extracted separately with acetone on Soxhlet's extraction apparatus for 8 hours. The extract was concentrated on rotary evaporator and finally made solvent free in a vacuum desicator.

Chemical fractionation of the extract, acetone extract of turmeric, was subjected to systematic chemical analysis by thin layer chromatography (TLC) on silica gel plate in a solvent system consisting of chloroform and acetone in 16:1 ratios which showed six clearly differentiable bands of six compound-fractions under ultraviolet light with different Rf values.

A glass column (60 cm length, 2.5 cm diameter) was packed with 85 gram silica gel slurry in the selected solvent system. One gram of the extract was partitioned in the column eluted with chloroform: acetone (16:1) to collect 533 fractions of 1 ml each. The TLC profile of every 3rd fraction was studied. On the basis of their TLC profiles the most nearly toning fractions were combined into six primary fractions on the basis of Rf values (Table 1, Figure 3). The 6th fraction was obtained by exhaustive elution of the remaining material from the column by solvent consisting of methanol and chloroform (1:1). This process was repeated twice to obtain sufficient amount of fractions for bioassays.

Evaluation of mortality and statistical analysis

Mortality was evaluated against fifty pairs of freshly emerged flies, introduced in separate rearing cages in three replications. These flies were fed upon 250 and 500 ppm of the turmeric acetone extract and three of its fractions F1, F3, and F6 (which showed the most promising repellence effects in preliminary studies) separately.

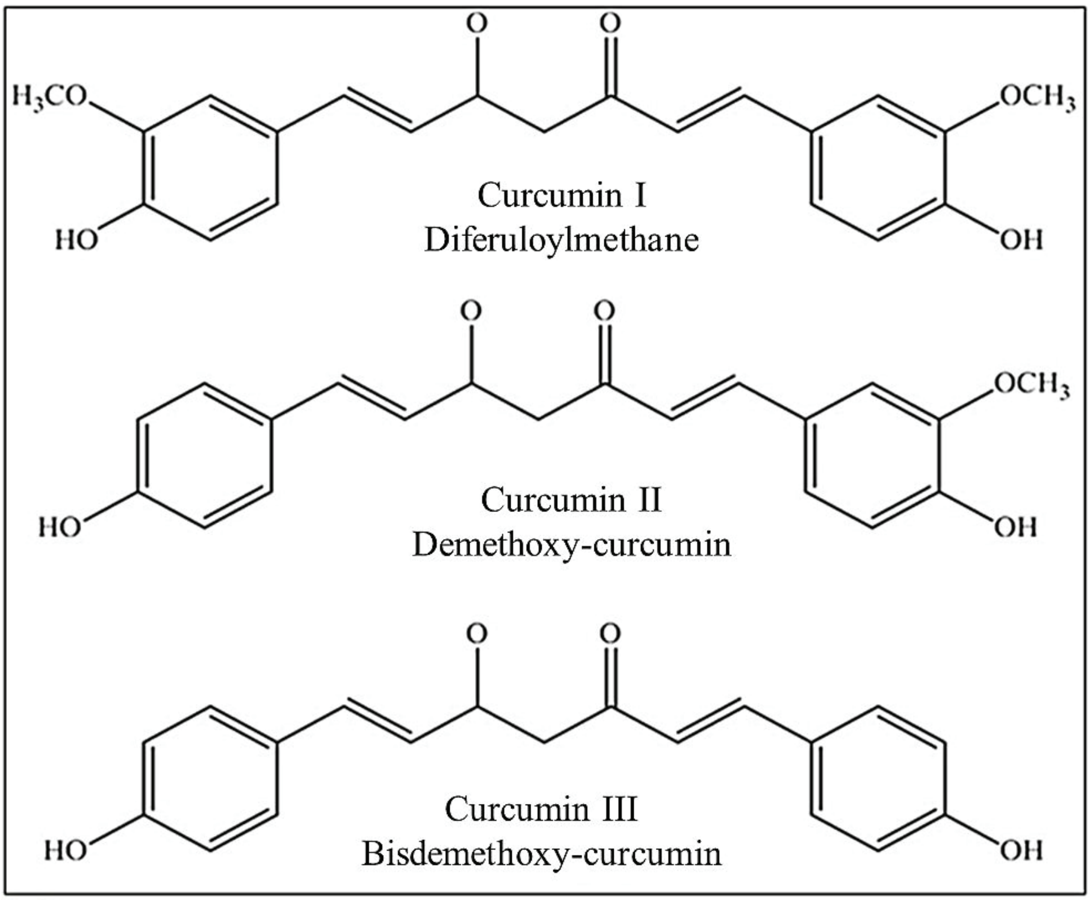

Figure 2: Structural formulae of Curcumin I (Diferuloylmethane), Curcumin II (Demethoxy-curcumin) and Curcumin III (Bisdemethoxy-curcumin).

Table 1: Chemical fractions of acetone extract of turmeric.

Fractions	Code	Rf value	Amount gms
1-117	F1	0.889	0.190
118-171	F2	0.778	0.310
172-210	F3	0.417	0.183
211-257	F4	0.278	0.225
258-441	F5	0.169	0.591
442-533	F6	0.056	0.447

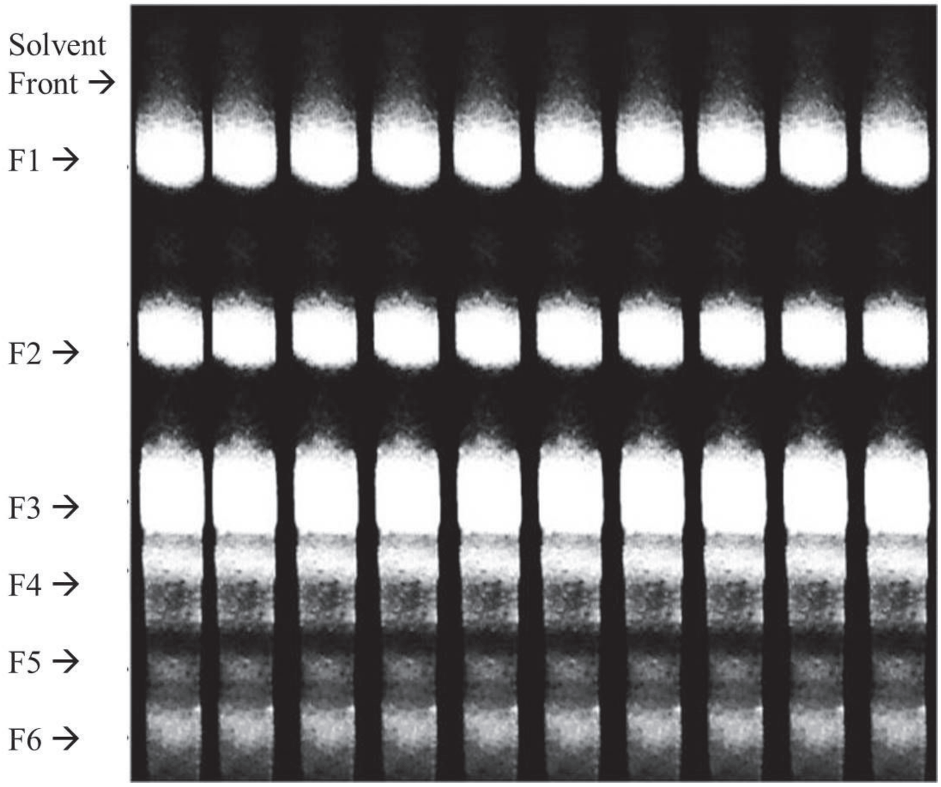

Figure 3: TLC profile of acetone extract of turmeric showing six different bands of fractions of Turmeric Extract (TE).

The extract and the fractions were mixed with artificial diet and fed for 20 days to the newly emerged females. Dead flies in each treatment were counted daily. Toxicity was evaluated by calculating the percent mortality after every five days interval by the formula given at Equation 1, below.

The data was recorded over 20 days. In this way the percent mortality was obtained for four different intervals of 1-5, 6-10, 11-15 and 16-20 days. The flies were also fed upon untreated diet similarly in another cage for comparison as control. Data so obtained was statistically analysed using Duncan's multiple range test.

$$\text{Percent Mortality} = \frac{\text{Number of dead flies over a time interval}}{\text{Total Number of flies introduced in the cage i.e 50}} \times 100 \quad (1)$$

RESULTS AND DISCUSSION

Mortality incurred by TE

In the first instance we carried out a toxicity assay using 250 and 500 ppm acetone extract of turmeric (TE) to find out if TE had any inhibitory effect on the flies. TE has been already studied for multiple effects such as repellence, oviposition deterrence, fecundity suppression and mortality against Peach fruit fly *Bactrocera zonata*. Flies fed on diet containing TE at concentrations 250 and 500 ppm showed significantly higher mortality than those fed on control (Siddiqi et al., 2011; Siddiqi et al., 2006; Rehman et al., 2009). TE caused significantly high mortality to flies compared to the flies fed on the normal diet, the control. The mortality also increased with the increasing concentration of TE, files fed at 250 ppm showed a mortality of 41.22 percent, significantly higher than 57.23 in control; at 500 ppm of TE fed in diet the mortality rose to 57.23 which was significantly higher than the mortality observed both in case of those fed at 250 ppm and on control, Table 2.

Mortality of flies over time

The percent mortality of flies fed on 500 ppm of TE in diet over four different time intervals is given in Table 3. It was observed that mortality was significantly different from each other over the four consecutive intervals of time. The mortality rose maximum during the third interval of 11-15days, the percent mortality at the end of 10th day was 31.55 which reached to 52.42 at the end of 15th day. During the 4th interval i.e. 16-20 days, the mortality observed was though significantly different but rose roughly by 2.8 % only i.e. from 52.42 to 55.24 .

Mortality of fractions of TE at 250 ppm

In our second trial we carried out a preliminary assay to calculate the percent mortality of flies fed on 250 ppm the TE, and its three fractions F1, F3, and F6 along with the control to find out which fraction of TE was most toxic for flies. The flies were fed with diet containing TE and its fractions and mortality was calculated on 20th day. The percent mortality for each of the five treatments was significantly different from each other. The maximum mortality 51.39 percent was observed for fraction F1 followed by 44.17 in case of TE. Similarly the mortality in case of fractions F3 and F6 was significantly higher than that of control but very lesser than that of F1 and TE. The fraction F1 of the TE was observed to be the most toxic component for flies, Table 4.

Table 2: Percent mortality of *B. zonata* after feeding on normal diet in control and the diet containing 250 and 500 ppm of acetone extract of turmeric in cage containing 50 pairs of fruit flies.

Feeding Ingredient ppm	Percent Mortality
TE 250	51.39B
TE 500	57.23A
Control	26.08C

Values followed by the same letters are not significantly different from each other ($p \leq 0.05$), having LSD value =1.549 Average of three replicates.

Table 3: Percent mortality of *B. zonata* during feeding for different time intervals on diet containing 500 ppm acetone extract in cage containing 50 pairs of female fruit flies.

Feeding Period (Days)	Percent Mortality
1-5	17.10D
6-10	31.55C
11-15	52.42B
16-20	55.24A

Values followed by the same letters are not significantly different from each other ($p \leq 0.05$), having LSD value =1.549 Average of three replicates.

Mortality of 500 ppm fractions of acetone extract

In the third trial we evaluated the percent mortality caused by 500 ppm of TE fractions F1, F3, F6, TE and control over fourth time intervals. Fraction F1 showed maximum percent mortality 70.37 and 72.22 during third and fourth intervals respectively which were significantly higher followed by that of turmeric extract TE 63.33 and 66.33 during the same intervals, Table 5. The overall mortality of the fractions, extract and control for all the four intervals is also given in Figure 4 which clearly manifests the fraction F1 to be highly toxic for flies followed by TE. The fractions F3 and F6 however, did not show considerable toxicity though significantly higher than that of control.

Table 4: Percent mortality of *B. zonata* after feeding on normal diet in control and the diet containing 250 ppm acetone extract and its three fractions in cage containing 50 pairs of fruit flies.

Feeding Ingredient	Percent Mortality
F1	51.39A
F3	38.42C
F6	35.33D
TE	44.17B
Control	26.08E

Values followed by the same letters are not significantly different from each other ($p \leq 0.05$), having LSD value =1.732 Average of three replicates.

Table 5: Percent mortality of *B. zonata* after feeding on normal diet in control and the diet containing 500 ppm acetone extract and its three fractions in cage containing 54 female fruit flies during various time intervals.

Turmeric Extract/ Fractions	Percent Mortality during different feeding periods (Days)			
	1-5	6-10	11-15	16-20
F1	22.22 I	40.74 .E	70.37 A	72.22 A
F3	18.51 J	35.18 F	50.00 C	50.00 C
F6	16.66 J	31.48GH	44.44 D	48.76 C
TE	17.00 J	30.00 H	63.33 B	66.33 B
Control	11.11 K	20.37 IJ	23.95 I	28.88 H

Values followed by the same letters are not significantly different from each other ($p \leq 0.05$), having LSD value =3.464 Average of three replicates.

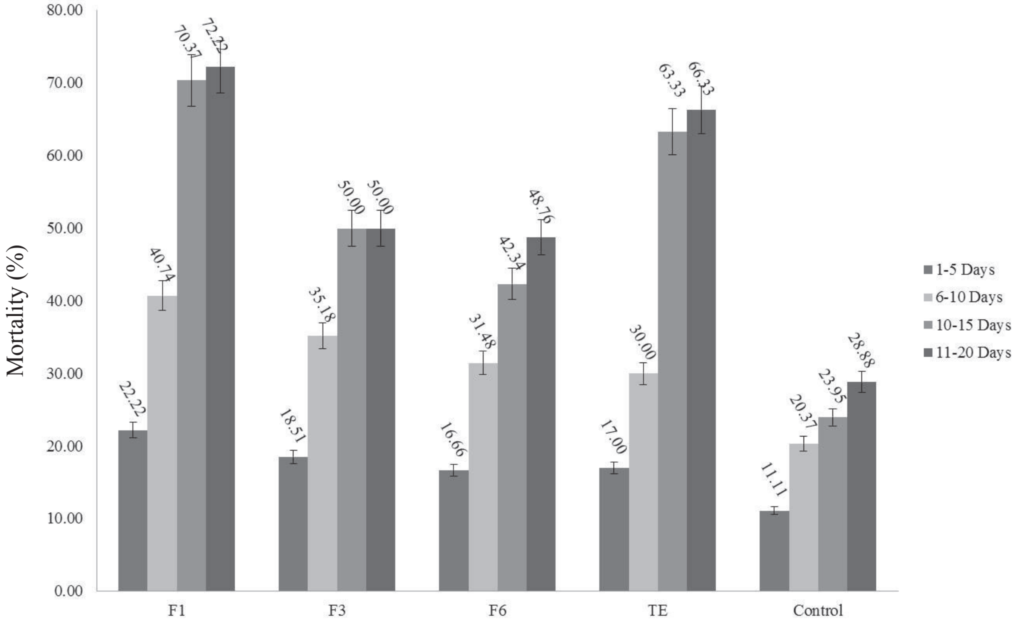

Figure 4: Percent mortality of fruit flies after feeding on normal diet as in control and the diet containing acetone extract of turmeric various time intervals.

CONCLUSIONS

In the management of fruit flies, major emphasis has been on the use of insecticides Out of total insecticides used in Pakistan; about 10 % is applied against fruit flies (Stonnehouse, et al., 1998). Most of the currently used insecticides are synthetic and non-selective which have caused serious social and environment repercussions. The toxic residues in agricultural products are not tolerable under the upcoming WTO regime (Enkerlin, 2007). The plants derivatives however, may be the potential source for the control of fruit flies with almost insignificant effects to environment and health. The acetone extract of turmeric is found to be highly toxic against fruit flies and its fraction F1 is its most toxic component which is primarily responsible for its potential toxicity. This study shows that turmeric has great potential to control fruit fly infestation, and is a prospective environment friendly natural insecticide candidate.

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